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PRELIMINARY REPORT ON AMINO ACID SYNTHESIS IN PLANTS

WALTER F. LOEHWING

INTRODUCTION

The process of amino acid synthesis in plants involves essentially a reduction of nitrates absorbed from the soil. It is difficult, however, to distinguish newly synthesized amino acids from those originating as hydrolytic cleavage products of proteins. To circumvent this difficulty, earlier investigators (2, 5, 6, 8), exposed their plants to nitrogen starvation, thereby reducing soluble nitrogen compounds in tissues to a minimum. Unfortunately, it is extremely difficult to remove final traces of nitrates and ammonia by starvation without causing high mortality in experimental material, and pathological conditions in many of the plants which do survive this rigorous treatment.

From the recent work of Murneek (6) concerning the correlative effects of fruits upon vegetative activity, the author obtained the idea that it might be possible to deprive tomato tissues of nitrates, ammonia and amino acids without subjecting the plants to severe nitrogen starvation by withholding all soil nitrates. Murneek has shown that developing tomato fruits deplete the nitrogen supply of the vegetative tissues, causing cessation of growth and ultimately death of the shoots due to lack of nitrogen. To test the feasibility of the plan, a preliminary set of small Bonny Best tomato plants were transplanted to pots filled with a sandy low-nitrogen soil. These plants were permitted to set fruit, and when signs of incipient nitrogen starvation were observed in leaves and stems, samples were taken from various parts of the plants, sectioned, and tested microchemically for nitrates, ammonia and amino acids. The vegetative tissues of the plants gave negative tests for the substances in question, although the plants were still fairly healthy.

Parenchyma cells were laden with carbohydrates, starch being abundant even in leaves throughout the day and night. It appeared, then, that by permitting developing tomato fruits to deplete the soluble nitrogenous products of stems and leaves it would be pos-

sible to follow with certainty the steps in the process of amino acid synthesis in relatively normal healthy plants.

METHODS

Plants of the Bonny Best variety of tomatoes, about eight inches tall, were removed from a loam to pots with a sandy soil low in nitrates. Plants were grown in a greenhouse and were watered at regular intervals with dilute nutrient solutions devoid of nitrogen. About two months later fruit had set, undeveloped flowers were falling off, new leaves remained small and incipient etiolation of lower leaves became apparent. Sections made from various parts of several plants were tested for (α) amino acids by orientation tests (10), nitrates by diphenylamine reagent (9), nitrites by sulph-anilic acid-alpha naphthylamine reagent (4), ammonia by Nessler's reagent (3), all of which were negative.

At the time developing fruits had induced nitrogen deficiency all fruits were picked from one-half the plants, and all plants supplied with calcium nitrate.

RESULTS

Twelve hours after the initial nitrate application, nitrates were detected in all parts of defruited plants except in the meristem of the tallest branches. Within twenty hours nitrates had reached the tips of the tallest shoots. This nitrate undoubtedly was that recently absorbed from the soil. Nitrites appeared in the stem tips and adjacent pericycle of defruited plants thirty to forty-eight hours after the addition of calcium nitrate.

Five to ten hours after nitrite appearance ammonia was detected at stem tips, followed shortly by positive tests for leucin and other amino acids. Colorimetric determination of hydrogen ion concentration (1) in stem sections revealed generally a distinctly acid central region (pith and xylem) for starch hydrolysis with a pH value between 4.6 and 5.0, and an alkaline periphery (phloem and cortical parenchyma) for nitrate reduction with a pH value of 7.2 to 8.0. As pointed out by Eckerson (2), however, sap extracted from such stem tips is strongly acid, indicating a rather rapid utilization or translocation of the amino acids formed there. The fact that this expressed sap, when made slightly basic, reduces nitrates, indicates that there is a reducing mechanism in these plants, important in amino acid synthesis which operates only in alkaline media. This reducing component of the sap is heat stabile which makes it appear that reduction is not a simple reductase phenomenon. Even though the alkaline region of stems is limited in area the above

mentioned facts demonstrate its importance in the reduction of nutrient nitrates.

Nitrogen metabolism in the fruiting plants differed somewhat from that above described for defruited plants. No positive tests for nitrates in tissues were obtained except in the cortex of roots and stem bases twelve to thirty hours after application of calcium nitrate to soil. Nitrites and amino acids appeared in shoot tops within twenty hours, while fruiting nodes were marked by abundance of amino acids in twenty-five to thirty hours. Alkalinity of the stem pericycle persisted in these fruiting plants just as in those defruited. Only a short growth period followed nitrate applications, showing the nitrogen deficiency was only temporarily alleviated in vegetative structures. Nitrogen was transported to fruits faster than it was absorbed. In these plants, nitrogen translocation was oriented to the fruits and the rate of nitrogen metabolism was much higher than in defruited plants. It is possible that fruits stimulate metabolism and translocation of nitrogen without causing an increase in rate of absorption resulting ultimately therefore in death of the plants on account of nitrogen exhaustion created by the fruit. Steps in the nitrate reduction process are similar to those previously reported by Eckerson (2).

CONCLUSIONS

It appears that fruit formation robs vegetative structures of normal tomato plant of nitrates, ammonia and amino acids. When defruited plants are supplied with nitrate fertilizer, the nitrates are absorbed as such accumulate at and are actively reduced in the vicinity of meristem, to nitrites, ammonia and amino acids. This reduction is most marked in alkaline tissues about the pericycle. In fact, if the behavior of the expressed sap be employed as a criterion, it appears that this alkalinity and the presence of sugars are indispensable for nitrate reduction and amino acid formation. The steps in the process, then, are: nitrates to nitrites to ammonia to amino acids in presence of sugars. Reduction is not enzymatic as boiled expressed sap reduces as efficiently as unboiled. Investigation is under way to determine the nature of the reducing agent, which is apparently bound up with the occurrence of carbohydrates.

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